



L7: Entry 1 of 4

File: USPT

Jul 4, 2000

DOCUMENT-IDENTIFIER: US 6083712 A

TITLE: Biotechnological method of producing biotin

## DEPR:

Cells of the biotin auxotrophic strain Agrobacterium/Rhizobium sp HK4 with the biotin producer plasmid pBO47 (DSM 8555) were cultured in a 2 l MBR fermenter in an L-glutamic acid/betaine minimal medium in a fed-batch method at 30.degree. C. until the OD<sub>sub.650</sub> was 70. HK4/pBO47 is characterized by a remarkably stable biotin synthesis rate even when growth is extremely slow ("maintenance growth"). For this reason, in this experiment the cultivation of the biomass was followed by a long maintenance phase (500 hours) with a greatly reduced carbon "feed".



L7: Entry 3 of 4

File: USPT

Nov 7, 1995

DOCUMENT-IDENTIFIER: US 5464763 A

TITLE: Process for the incorporation of foreign DNA into the genome of dicotyledonous plants

## DEPR:

Since this plasmid cannot replicate in *A. tumefaciens* bacteria, the plasmid has been converted into a plasmid having a broad host range by fusion with the IncP plasmid R772. For this purpose R772 was introduced into the strain HB101 (with plasmid POTY8) by conjugation, whereupon transconjugants of this crossing were used as donors in further crossings with the *A. tumefaciens* strains LBA202. Transconjugants hereof were selected for the presence of the ampicillin resistance marker of POTY8. As was expected, these strains would contain a cointegrate plasmid of POTY8 and R772, because POTY8 itself is not conjugative and cannot replicate in Agrobacterium. The introduction of R772 could have taken place either in the vector part or the T-region part of POTY8. In order to be able to carry out complementation experiments, only a cointegrate containing an intact T-region is of importance. That is why subsequently 30 transconjugants were conjugated with the *E. coli* strain JA221 (C600 trpE leu B, *vide* Beggs, *Nature* 275, 104-109 (1978)), whereupon the progeny was examined for leucin auxotrophy. One of the 30 transconjugant strains appeared not to grow on a minimum medium without leucin added. Probably, this strain contained a R772:: POTY8 cointegrate plasmid, in which the expression of the gene LEU-2 had been inactivated by the incorporation of R772. Analysis of restriction endonuclease patterns of the R772:: POTY8 plasmid, which was called pAL1050, showed that the plasmid pAL1050 had an insertion of R772 in the pJDB207 part of POTY8, whereas the T-region had remained unmodified. The structural organisation was further confirmed by hybridisation experiments using the Southern blot technique (Southern, *J. Mol. Biol.* 98, 503-518 (1975)) and of labelled plasmid DNA of R772 and POTY8. The plasmid pAL1050 and the way in which it is manufactured, are shown in outline in FIG. 1. Herein the T-region is indicated in shading. One of the two copies of the insertion sequence IS70 got partly lost, which accounts for the surprising stability of the cointegrate plasmid pAL1050 found.



69|667,796

L1: Entry 2 of 157

File: USPT

Oct 9, 2001

DOCUMENT-IDENTIFIER: US 6300545 B1

TITLE: Mobilization of viral genomes from T-DNA using site-specific recombination systems

## DEPR:

To infect plant cells, virulence (vir) gene activity is induced by treatment with acetosyringone in Agrobacterium carrying the vector constructed in Example 1. Agrobacterium cells are grown to a density of  $2 \times 10^9$  cells per ml (A=100, using a Klett-Summerson spectrophotometer, red filter) in AB-sucrose medium. The cells are centrifuged at 10,000 g, suspended at a concentration of  $1 \times 10^9$  cells per ml (A=50) in induction medium (AB salts, 0.5% glucose, 2 mm sodium phosphate, 50  $\mu$ M Mes, Ph 5.6, 50 FM acetosyringone), and incubated with gentle shaking at 25°C. for 14 to 18 hr. After washing the bacterial cells in plant culture medium, plant cells are inoculated with induced Agrobacterium (-20 bacterial cells per plant cell, except where noted otherwise) and cocultivated at 25°C. with shaking at 140 rpm for various periods of time. Most of the bacteria is washed off by centrifugation of the cocultivation mixture at 300 rpm (model GLC-2 clinical centrifuge; Beckman Sorvall, Newtown, Conn.) for 2 min. The plant cell pellet is suspended and washed once more in plant culture medium and then resuspended in culture containing either 100  $\mu$ g/ml timentin or 200  $\mu$ g/mL cefotaxime. Mobilization of viral genomes from the T-DNA is monitored by quantitating infectious viral particles.

L7: Entry 2 of 4

File: USPT

Jan 12, 1999

DOCUMENT-IDENTIFIER: US 5858759 A

TITLE: D-N-carbamoyl-amino acid amidohydrolase and hydantoinase

DEPR:

The method used for this transfer was very similar to that described for pCAR1 in Example 12. The parents in the mating were E. coli HB101 (pCAR6)--see Example 5, E. coli HB101 (pRK2013)--see Example 12--and Agrobacterium 15-10--see Example 12. Since both E. coli parent strains are auxotrophs, the progeny from the mating were plated on MM containing 10 .mu.g/ml tetracycline, but no streptomycin, in order to select for Agrobacterium 15-10 (pCAR6) colonies. The presence of pCAR6 was confirmed by analysis of restriction enzyme digestion of plasmid DNA isolated from ex-conjugants.



L1: Entry 1 of 157

File: USPT

Oct 16, 2001

DOCUMENT-IDENTIFIER: US 6303341 B1

TITLE: Method for producing immunoglobulins containing protection proteins in plants and their use

DEPR:

Cotyledon pieces of approximately 0.5 cm diameter were cut with a sterile scalpel and placed on agar plates containing MS4 medium (MS4 medium per liter: 4.4 g Murashige and Skoog basal salts with minimal organics [Sigma #M68991, 30 g sucrose, 2 mg zeatin riboside, 5 mg nicotinic acid, 0.5 mg pyridoxin, 0.5 mg thiamine, 1 mM acetosyringone, 10 g agar, pH 5.7 with KOH]).

DEPR:

To the leaf pieces was then added 2 ml of a suspension of Agrobacterium in LB (approximately 1.times.10.<sup>8</sup> Agrobacteria per ml). All surfaces of the leaf discs were contacted with Agrobacteria, excess liquid was poured off the plate, and the discs were co-cultivated with the bacteria for 2 days at room temperature. The discs were then transferred to agar plates containing MS4 medium minus acetosyringone containing 50 .mu.g/ml kanamycin and 250 .mu.g/ml carbenicillin (MS4-KC). Regeneration was allowed to proceed with weekly transfer of discs to fresh MS4-KC plates until regenerating shoots were visible. Shoots were then transferred to agar plates containing MSO-KC medium (MSO-KC per liter: 4.4 g Murashige and Skoog basal salts with minimal organics [Sigma #M68991, 30 g sucrose, 1 mg nicotinic acid, 1 mg pyridoxin, 10 mg thiamine, 50 .mu.g/ml kanamycin and 250 .mu.g/ml carbenicillin, 10 g agar, pH 5.7 with KOH]).

**End of Result Set** **Generate Collection**

L3: Entry 2 of 2

File: USPT

Apr 24, 1990

DOCUMENT-IDENTIFIER: US 4918863 A

TITLE: Method and apparatus for planting seeds and growing plants

**DEPR:**

Although herein described for outdoor application, the method is equally applicable for indoor and greenhouse use. For such use, virgin soil 12 may be transported to form a growing bed. Or, other types of plant nutrient medium beds may be provided, such as are common for greenhouse use. To provide for re-use of the enclosure group 10, the paper may be impregnated or coated, as, for example, with epoxies. Or, it may be constructed of decay resistant plastic, especially applicable for repeated greenhouse use. A material of woven fabric could also be used. Also, while the collapsible groups 10c (FIGS. 7-10) is quite desirable for storage and transport, a non-collapsible matrix of enclosures may also be employed, of plastic for example or of non-woven fabric such as paper, without departure from the spirit of the invention. The illustrated manual planting of the seeds may of course be replaced by planting with appropriate tools or devices without departing from the spirit of the inventive method. Also, other methods of treatment of the material of the enclosure group 10 may be desirable, including treatment with fertilizing material, or growth promoters or inhibitors such as hormones, other growth regulators, and germination stimulants. Examples of fertilizers include potassium nitrate or sulfate, controlled availability fertilizers such as urea-formaldehyde, ammonium phosphate, super phosphate and others. Germination and/or root growth promoters include the gibberellines, kinetin, *b*-benzylaminopurine, PBA, indoleacetic acid, napthaleneacetic acid, phenoxy compounds (2-4D) and others.

L3 ANSWER 6 OF 43 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.  
AN 2000:254077 BIOSIS  
DN PREV200000254077  
TI Study of the factors influencing Agrobacterium-mediated transformation of pea (*Pisum sativum* L.).  
AU Nadolska-Orczyk, Anna (1); Orczyk, Waclaw  
CS (1) Plant Breeding and Acclimatization Institute, Radzikow, 05-870, Blonie Poland  
SO Molecular Breeding, (April, 2000) Vol. 6, No. 2, pp. 185-194. print..  
ISSN: 1380-3743.  
DT Article  
LA English  
SL English  
AB Factors influencing the efficiency of Agrobacterium-mediated transformation of pea were tested using highly efficient, direct regeneration system. The virulence of three Agrobacterium strains (octopine LBA 4404, nopaline C58C1 and succinamopine, hypervirulent **EHA 105**) clearly varied giving 1 transgenic plant per 100 explants for LBA 4404, 2.2 for C58C1 and 8.2 for **EHA 105**. To test the efficacy of selection agents we used the hypervirulent **EHA 105** strain carrying pGPTV binary vector with one of four different selection genes: nptII, hpt, dhfr or bar. The mean number of transgenic, kanamycin-resistant plants for two cultivars tested was 4.2 per 100 explants and was slightly higher than the number of phosphinothricin-resistant plants (3.6 plants per 100 explants). The proportion of transgenics among kanamycin-selected plants was also higher than among phosphinothricin-resistant plants (35% and 28% respectively). There was no regeneration on hygromycin or methotrexate media (transformation with hpt and dhfr genes). Acetosyringone had no apparent influence on efficiency of transformation with hypervirulent **EHA 105** strain, however it did affect the rate of transformation when moderately virulent C58C1 was used. Recovery of transgenic plants was enhanced after application of 5-azacytidine. The presence of integrated T-DNA was checked by PCR and confirmed by Southern hybridization. T-DNA was stably transmitted to the next generation.  
CC Genetics and Cytogenetics - Plant \*03504  
Genetics of Bacteria and Viruses \*31500  
Plant Physiology, Biochemistry and Biophysics - Apparatus and Methods \*51524

AN 2000:202793 BIOSIS

DN PREV200000202793

TI Efficient production of transgenic citrus plants expressing the coat protein gene of citrus tristeza virus.

AU Dominguez, A.; Guerri, J.; Cambra, M.; Navarro, L.; Moreno, P.; Pena, L.  
(1)

CS (1) Dpto. Proteccion Vegetal y Biotecnologia, Instituto Valenciano de Investigaciones Agrarias (IVIA), E-46113, Moncada, Valencia Spain

SO Plant Cell Reports, (March, 2000) Vol. 19, No. 4, pp. 427-433.

ISSN: 0721-7714.

DT Article

LA English

SL English

AB The coat protein gene of citrus tristeza virus (CTV) has been introduced into Mexican lime (*Citrus aurantifolia* Swing.) plants by using an improved Agrobacterium-mediated genetic transformation system. Internodal stem segments from greenhouse-grown seedlings were co-cultivated with *A. tumefaciens* strain **EHA 105** carrying the binary plasmid pBI 121/CTV-CP in a medium rich in auxins that provided the explant cells with the proper treatment to shift them to a competent state for transformation. The transformation frequency was enhanced, and this allowed us to recover 42 transgenic plants from 1200 explants. Regenerated shoots were identified as transformants by performing beta-glucuronidase (GUS) assays and subsequently by PCR amplifications of the CTV-CP transgene. Southern analyses revealed that at least one copy of the CTV-CP gene was integrated in all PCR positive plants. Interestingly, 70% of them had linked T-DNAs arranged at one locus. Copy number of the CTV-CP gene varied from one to six among the transgenic lines. Half of them showed truncated T-DNAs in which the left border was lost. Expression of the CTV-CP transgene was demonstrated in 38 out of 42 plants by western analysis and DASI-ELISA. No correlation was found between coat protein expression and transgene copy number or integration pattern.CC Horticulture - Tropical and Subtropical Fruits and Nuts; Plantation Crops  
\*53004

Genetics and Cytogenetics - Plant \*03504

Genetics of Bacteria and Viruses \*31500

BC Closterovirus 02808

Rutaceae 26685

AN 1998:404637 BIOSIS

DN PREV199800404637

TI Studies of development of efficient genetic transformation system and factors that affect transformation of the commercial apple cultivars.

AU Zhang, Zhi-Hong; Fang, Hong-Jun (1); Jing, Shi-Xi (1); Wang, Guan-Lin; Wu, Lu-Pin; Zhu, Zhen (1)

CS (1) Dep. Pomol., Shenyang Agric. Univ., Shenyang 110161 China

SO Acta Genetica Sinica, (1998) Vol. 25, No. 2, pp. 160-165.

ISSN: 0379-4172.

DT Article

LA Chinese

SL Chinese; English

AB Several factors that affect genetic transformation of the commercial apple, cultivars were examined. A simple and efficient genetic transformation system was developed. The efficiency of exogenous genes transferring into cells of apple cultivar New Jonagold was obviously higher than the efficiency of exogenous genes transferring into cells of apple cultivar Royal Gala or Fuji. The ability of strain **EHA** 105-mediated gene transfer was higher remarkably than the ability of strain LBA 4404. Inducing vir gene expression factors, such as acetosyringone, phosphate starvation and low pH, had no obvious effect on the efficiency of strain EHA105-mediated transformation. The time of dipping leaf pieces in suspension affected not only the gene transfer efficiency, but also the inhibition by cefotaxime of the growth of Agrobacterium on explants. The transformation efficiency of leaf pieces which abaxial side contacted with medium during concultivation was higher than the efficiency of leaf pieces which adaxial side contacted with medium.

CC Genetics and Cytogenetics - General \*03502

Genetics and Cytogenetics - Plant \*03504

Genetics of Bacteria and Viruses \*31500

BC Rhizobiaceae 06509

Rosaceae 26675

IT Major Concepts

Genetics; Methods and Techniques

IT Parts, Structures, &amp; Systems of Organisms

leaf: abaxial side, adaxial side

IT Methods &amp; Equipment

genetic transformation system: DNA transfer method, efficiency

ORGN Super Taxa

Rhizobiaceae: Gram-Negative Aerobic Rods and Cocci, Eubacteria, Bacteria, Microorganisms; Rosaceae: Dicotyledones, Angiospermae, Spermatophyta, Plantae

ORGN Organism Name

Agrobacterium-tumefaciens (Rhizobiaceae): strain-EHA105, strain-LBA4404; Malus-domestica [apple] (Rosaceae): commercial cultivar, transgenic, cultivar-Fuji, cultivar-New Jonagold, cultivar-Royal Gala

ORGN Organism Superterms

Angiosperms; Bacteria; Dicots; Eubacteria; Microorganisms; Plants; Spermatophytes; Vascular Plants

=> file ca

ATPL (CHNFT

=> s (auxotroph? (10a) agrobacterium) /ab,bi

L1 7 (AUXOTROPH? (10A) AGROBACTERIUM) /AB,BI

=> file biosis

=> s 11

L2 10 (AUXOTROPH? (10A) AGROBACTERIUM) /AB,BI

=> dup rem

L3 14 DUP REM L1 L2 (3 DUPLICATES REMOVED)

=> d l3 1-14 ti py

=> d l3 ab 1-2 6-7

L3 ANSWER 6 OF 14 CA COPYRIGHT 2003 ACS

AB With the increased use of plasmid Ti-based vectors and *A. tumefaciens* as their bacterial host for plant genetic engineering, concern has been expressed regarding their containment problems. For example, if a strain of *A. tumefaciens* with a recombinant Ti plasmid contg. a DNA fragment from a pathogenic virus escapes from the lab., it might become a serious, man-made pest with a wide host range. Use of tryptophan auxotrophs of *A. tumefaciens* might obviate this problem; such strains lose their tumorigenic ability but regain it upon reversion to prototrophy. Perhaps bacterium-produced auxin is essential for the onset of tumorigenesis, and its prodn. ceases due to the lack of tryptophan. The presence of a mutation called ctu causes a tryptophan auxotroph to remain nontumorigenic even if it reverts to tryptophan prototrophy.

=> d l3 bib 1 2 6 7

L3 ANSWER 6 OF 14 CA COPYRIGHT 2003 ACS

Q K 981. P57

AN 106:2713 CA

TI Tryptophan \*\*\*auxotrophs\*\*\* for increasing safety of \*\*\*Agrobacterium\*\*\* Ti-based recombinant plasmid work

AU Sastry, G. R. K.; Miles, Carol A.; Miller, Ian S.; Borland, Patricia A.; Saeed, Nasim; May, Christine A.

CS Dep. Genet., Univ. Leeds, Leeds, LS2 9JT, UK

SO Plant Molecular Biology Reporter (1986), 4(2), 93-7  
CODEN: PMBRD4; ISSN: 0735-9640

DT Journal

LA English

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